# Analysis of Quantitative data

# Student's *t*-test

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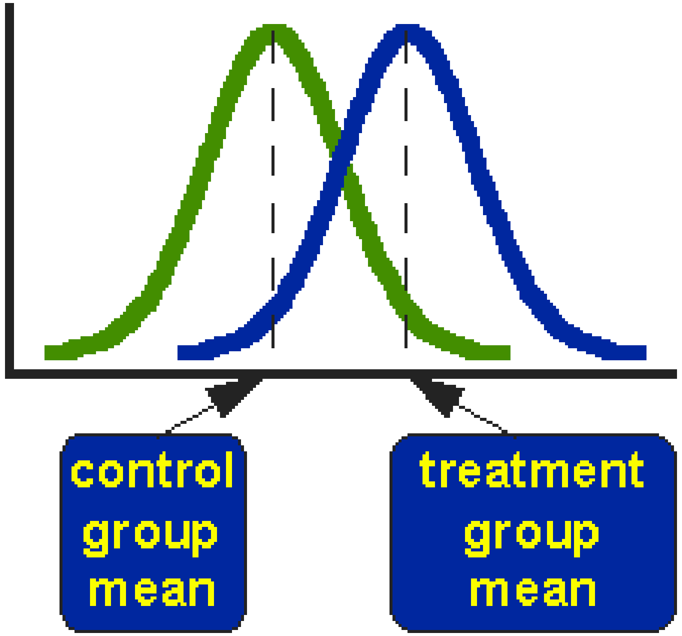
# Comparison between 2 groups

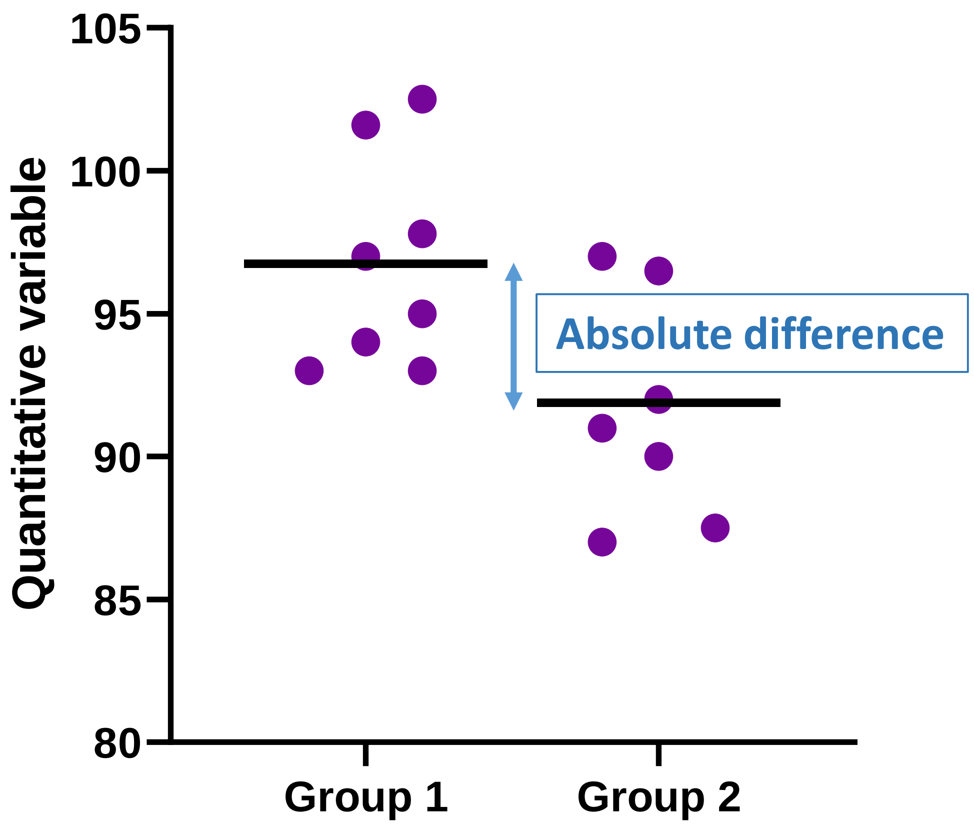
# Comparison between 2 groups:

## Student's *t*-test

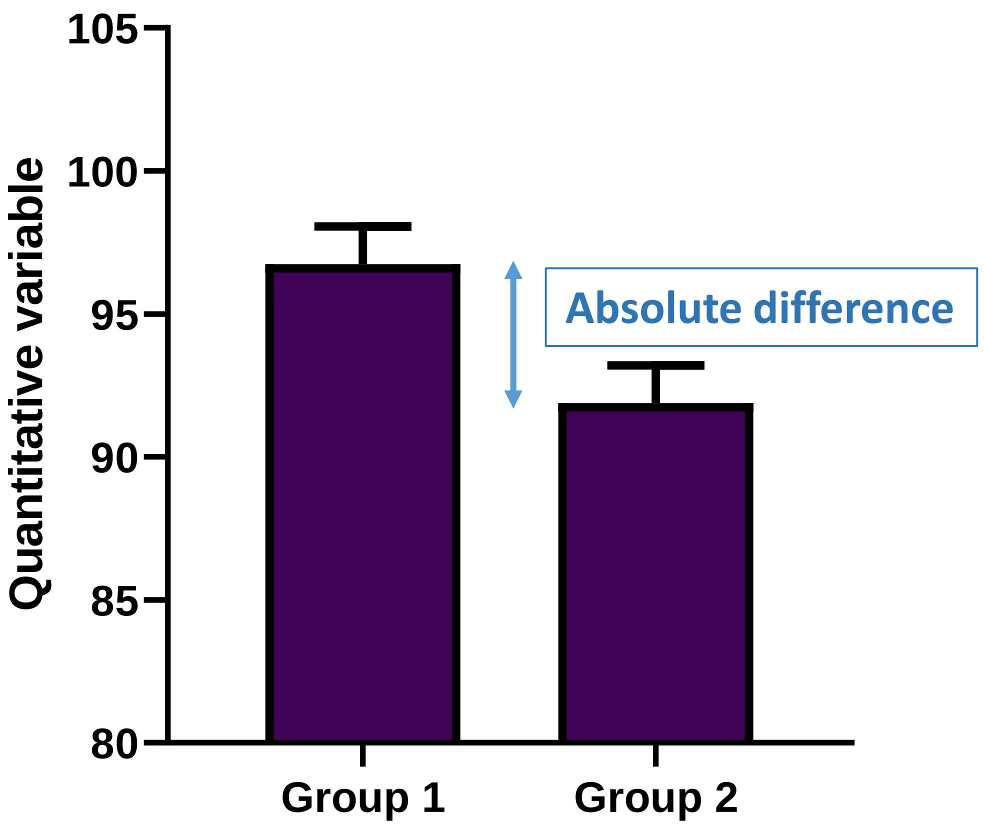
- **Basic idea**:
  - When we are looking at the differences between scores for 2 groups, we have to judge the difference between their means relative to the spread or variability of their scores.
    - Eg: comparison of 2 groups: control and treatment



# Variability does matter

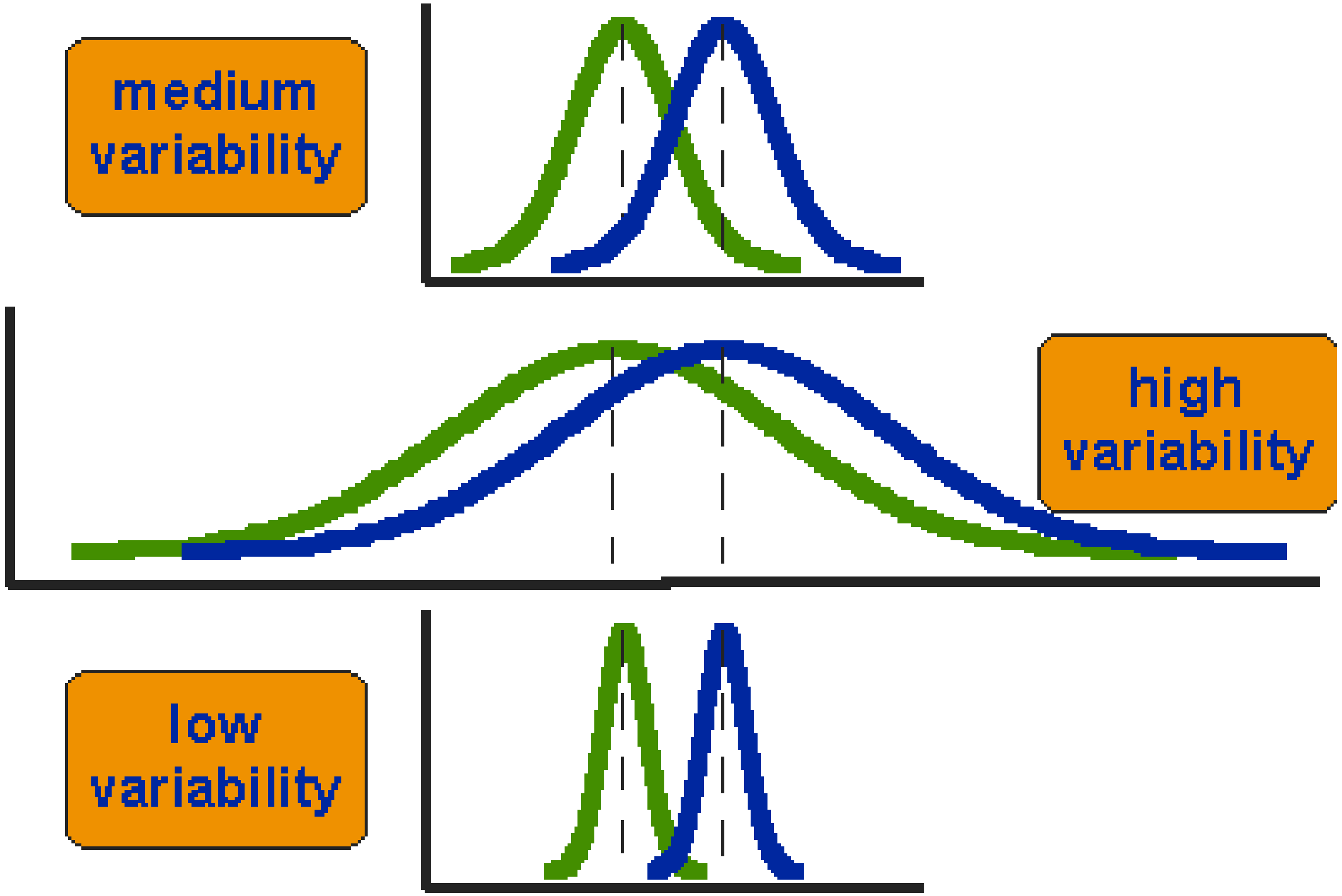



Scatter plot




Bar chart

# Student's *t*-test

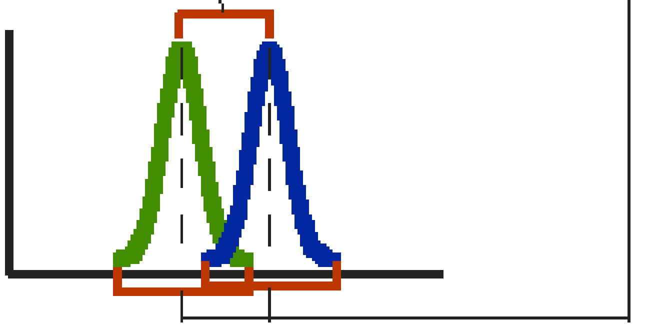


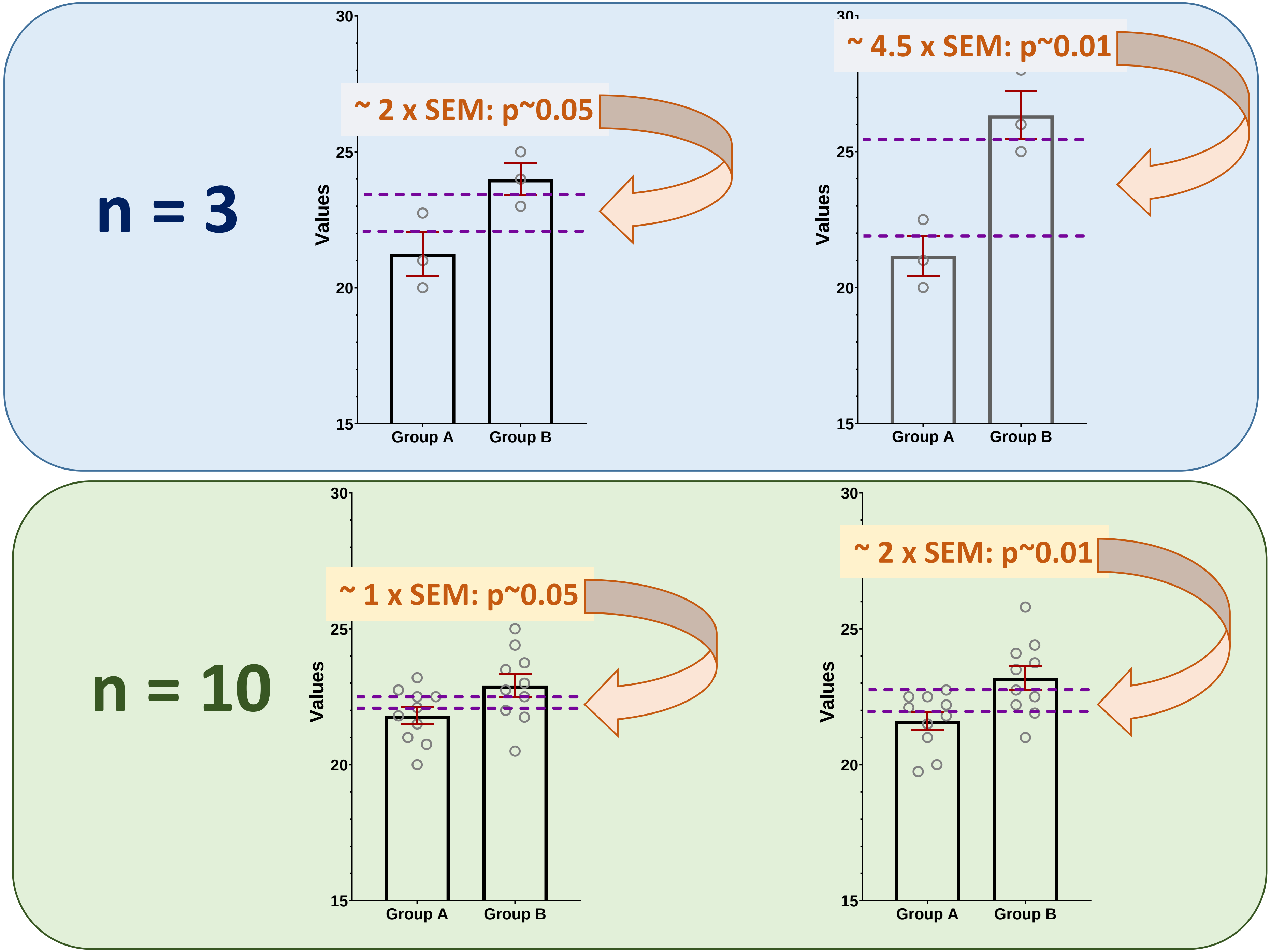
# Student's *t*-test

$$\frac{\text{signal}}{\text{noise}} = \frac{\text{difference between group means}}{\text{variability of groups}}$$

$$= \frac{\bar{X}_T - \bar{X}_C}{\sqrt{\frac{var_T}{n_T} + \frac{var_C}{n_C}}}$$

$$= \text{t-value}$$





n = 3
~ 2 x SEM: p~0.05
~ 4.5 x SEM: p~0.01
n = 10
~ 1 x SEM: p~0.05
~ 2 x SEM: p~0.01
Values
Group A
Group B
15
20
25
30

# Student's *t*-test

- **Independent t-test**
  - Difference between 2 means of one variable for <u>two independent groups</u>
    - Example: difference in weight between WT and KO mice

- **Paired t-test**
  - Difference between two measures of one variable for <u>one group</u>:
    - Example: before-after measurements
      - the second 'sample' of values comes from the same subjects (mouse, petri dish …).
    - Importance of experimental design!

- One-Sample t-test
  - Difference between the mean of a single variable and a specified constant.

# Example: coyotes.xlsx

- Question: do male and female coyotes differ in size?

- **Sample size**
- **Data exploration**
- **Check the assumptions for parametric test**
- **Statistical analysis: Independent t-test**

# Exercise: Power analysis

- Example case:

No data from a pilot study but we have found some information in the literature.

In a study run in similar conditions as in the one we intend to run, **male coyotes** were found to measure: **92cm+/- 7cm (SD)**.

We expect a **5% difference** between genders.

- **smallest biologically meaningful difference**

# G*Power

## Independent t-test

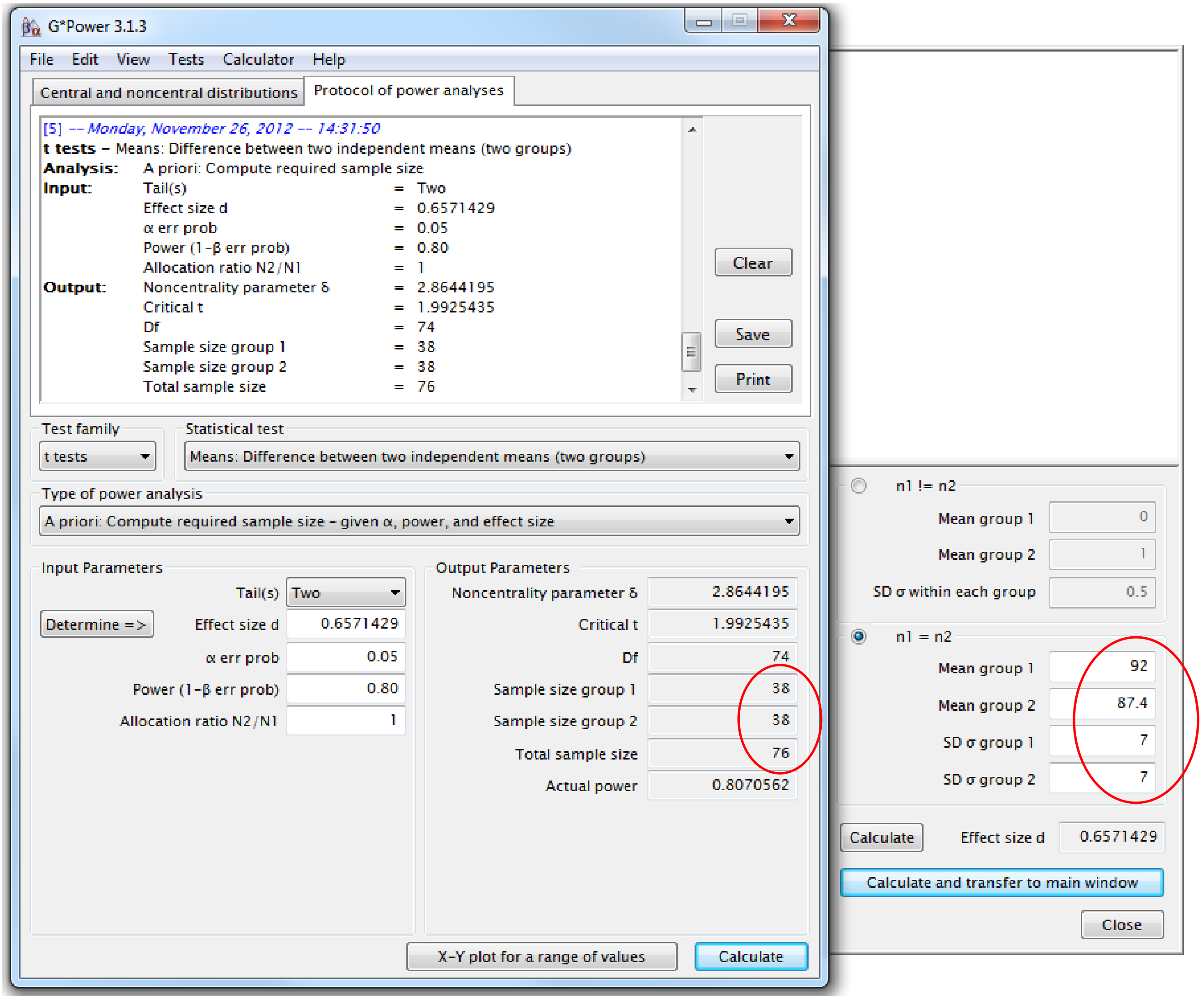
**A priori Power analysis**

Example case:

You don't have data from a pilot study but you have found some information in the literature.

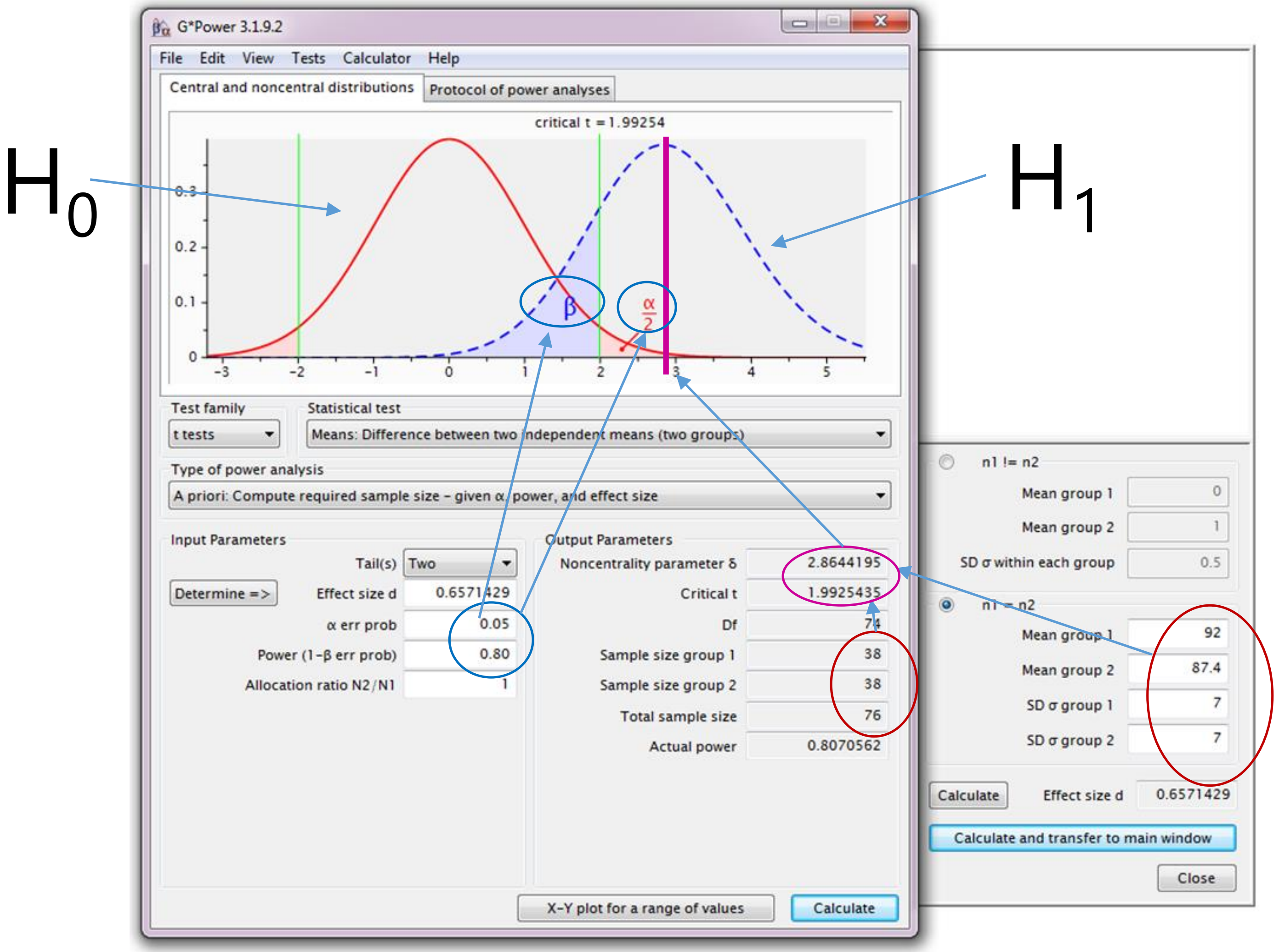
In a study run in similar conditions to the one you intend to run, male coyotes were found to measure:
92cm+/- 7cm (SD)

You expect a 5% difference between genders with a similar variability in the female sample.



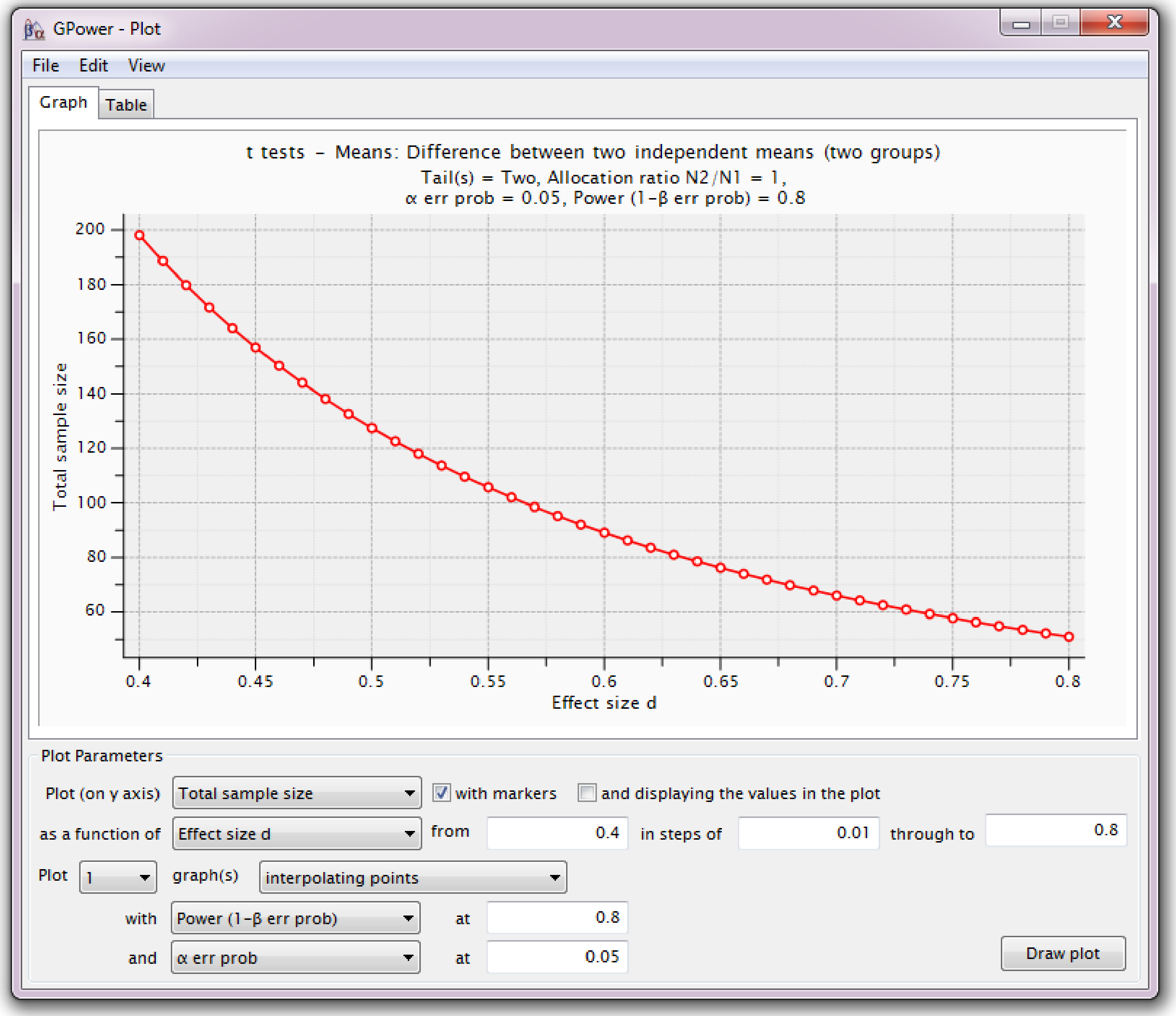

**You need a sample size of n=76 (2*38)**

# Power Analysis



# Power Analysis

For a range of sample sizes:



# Data exploration $\neq$ plotting data

# Exercise: Data exploration

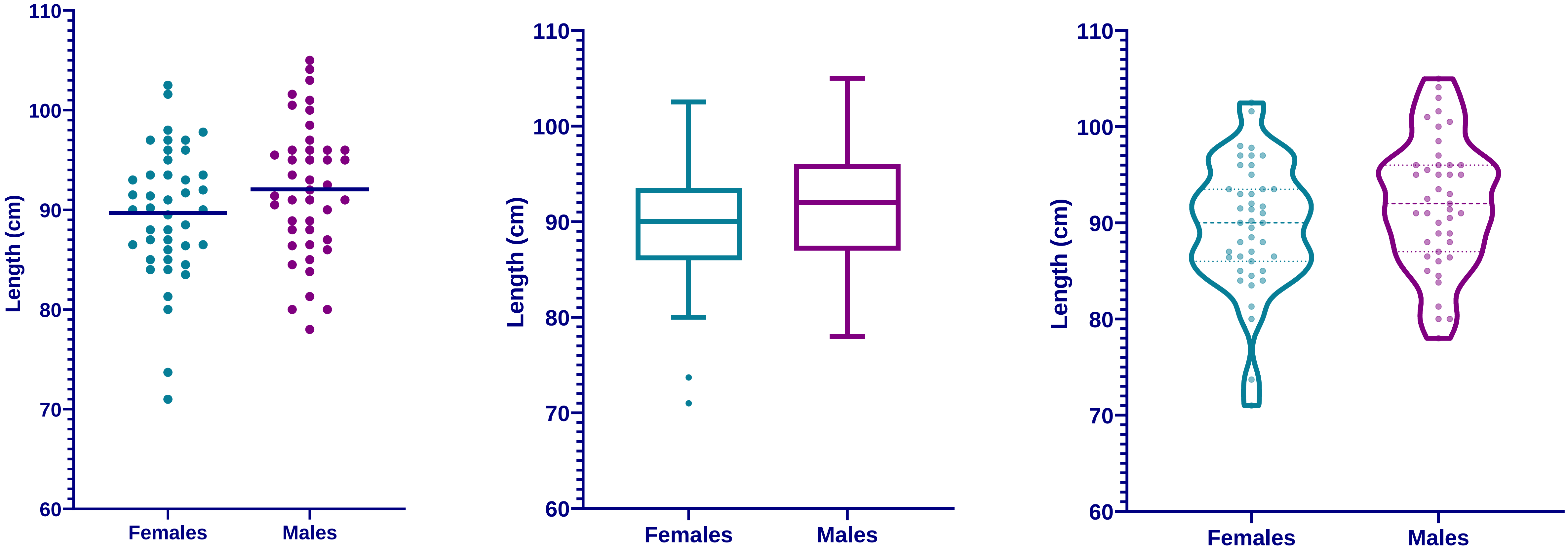
## coyotes.xlsx

- The file contains individual body length of male and female coyotes.

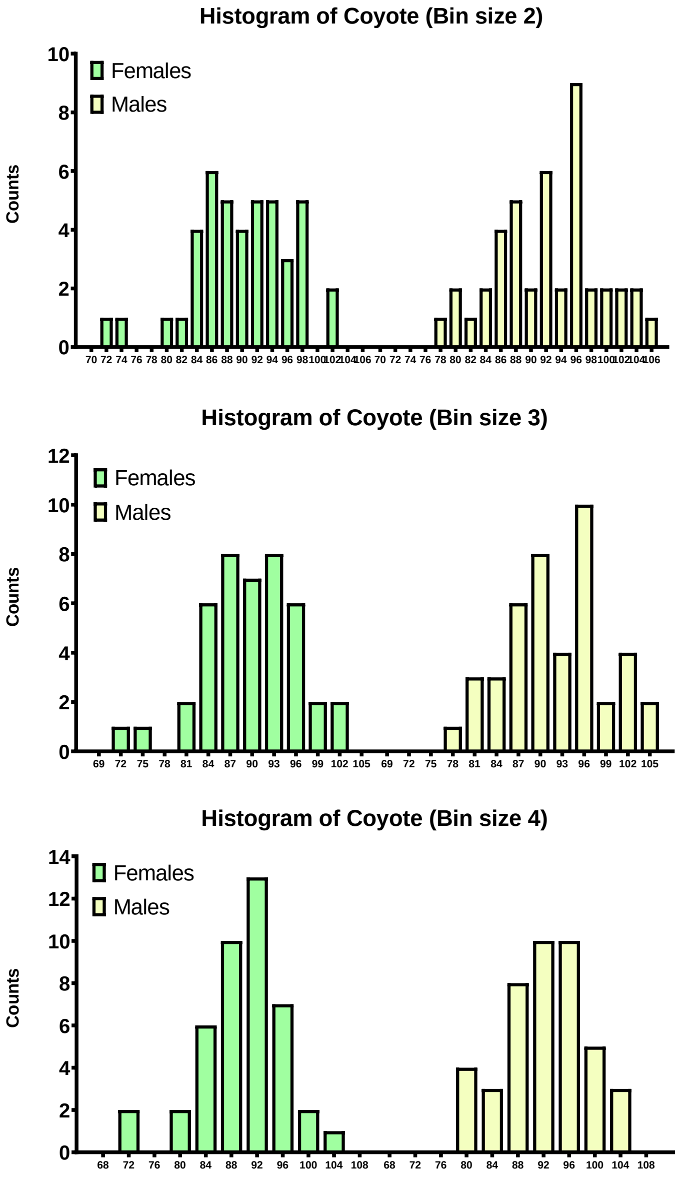
Question: do male and female coyotes differ in size?

  - Plot the data as stripchart, boxplot and violinplot

# Exercise: Exploring data - *Answers*



# Assumptions for parametric tests




**Normality ☑**

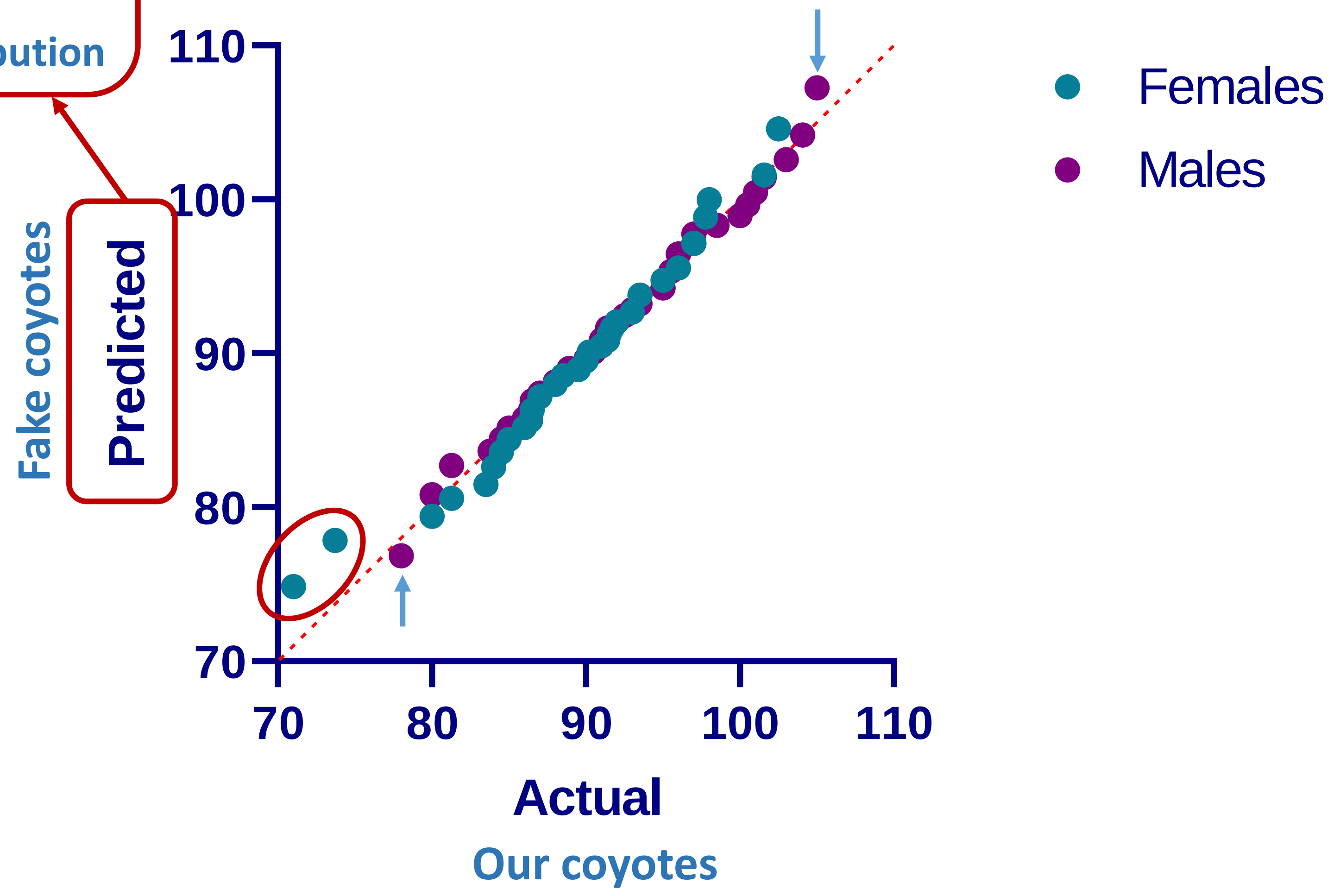
**Normality and Lognormality Tests**
Tabular results

| | | A Females | B Males |
|---|---|---|---|
| 1 | **Test for normal distribution** | | |
| 2 | **Anderson-Darling test** | | |
| 3 | A2* | 0.3158 | 0.1750 |
| 4 | P value | 0.5294 | 0.9192 |
| 5 | Passed normality test (alpha=0.05)? | Yes | Yes |
| 6 | P value summary | ns | ns |
| 7 | | | |
| 8 | **D'Agostino & Pearson test** | | |
| 9 | K2 | 4.203 | 0.5080 |
| 10 | P value | 0.1223 | 0.7757 |
| 11 | Passed normality test (alpha=0.05)? | Yes | Yes |
| 12 | P value summary | ns | ns |
| 13 | | | |
| 14 | **Shapiro-Wilk test** | | |
| 15 | W | 0.9700 | 0.9845 |
| 16 | P value | 0.3164 | 0.8190 |
| 17 | Passed normality test (alpha=0.05)? | Yes | Yes |
| 18 | P value summary | ns | ns |
| 19 | | | |
| 20 | **Kolmogorov-Smirnov test** | | |
| 21 | KS distance | 0.07845 | 0.08853 |
| 22 | P value | >0.1000 | >0.1000 |
| 23 | Passed normality test (alpha=0.05)? | Yes | Yes |
| 24 | P value summary | ns | ns |
| 25 | | | |
| 26 | **Number of values** | 43 | 43 |

# Normality assumption

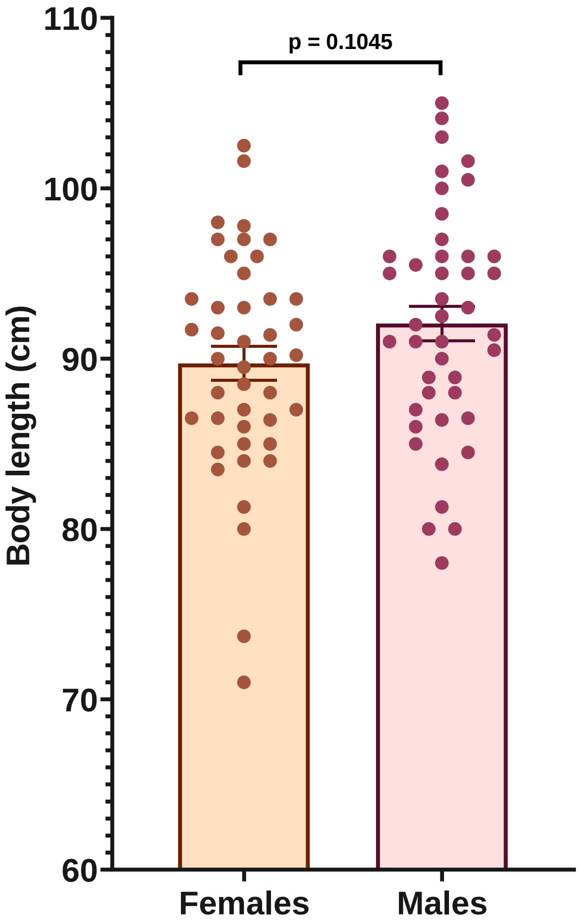
Same mean
Same SD
Same sample size
BUT
perfectly normal distribution

Normal QQ plot = Quantile – Quantile plot



# Independent *t*-test: results

| | Unpaired t test | |
|---|---|---|
| 1 | Table Analyzed | Coyote |
| 2 | | |
| 3 | Column A | Females |
| 4 | vs. | vs. |
| 5 | Column B | Males |
| 6 | | |
| 7 | **Unpaired t test** | |
| 8 | P value | 0.1045 |
| 9 | P value summary | ns |
| 10 | Significantly different (P < 0.05)? | No |
| 11 | One- or two-tailed P value? | Two-tailed |
| 12 | t, df | t=1.641, df=84 |
| 13 | | |
| 14 | **How big is the difference?** | |
| 15 | Mean of column A | 89.71 |
| 16 | Mean of column B | 92.06 |
| 17 | Difference between means (A - B) ± SEM | -2.344 ± 1.428 |
| 18 | 95% confidence interval | -5.185 to 0.4964 |
| 19 | R squared (eta squared) | 0.03107 |
| 20 | | |
| 21 | **F test to compare variances** | |
| 22 | F, DFn, Dfd | 1.045, 42, 42 |
| 23 | P value | 0.8870 |
| 24 | P value summary | ns |
| 25 | Significantly different (P < 0.05)? | No |
| 26 | | |
| 27 | **Data analyzed** | |
| 28 | Sample size, column A | 43 |
| 29 | Sample size, column B | 43 |
| 30 | | |



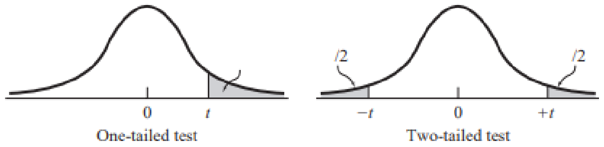

**Homogeneity in variance ☑**

**Males tend to be longer than females but not significantly so (p=0.1045)**

# Independent *t*-test: results

## *The old-fashion way*

| Unpaired t test | |
|---|---|
| P value | 0.1045 |
| P value summary | ns |
| Significantly different (P < 0.05)? | No |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=1.641, df=84 |




| | Level of Significance for One-Tailed Test | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.25 | 0.20 | 0.15 | 0.10 | 0.05 | 0.025 | 0.01 | 0.005 | 0.0005 |
| | Level of Significance for Two-Tailed Test | | | | | | | | |
| df | 0.50 | 0.40 | 0.30 | 0.20 | 0.10 | 0.05 | 0.02 | 0.01 | 0.001 |
| 1 | 1.000 | 1.376 | 1.963 | 3.078 | 6.314 | 12.706 | 31.821 | 63.657 | 636.620 |
| 2 | 0.816 | 1.061 | 1.386 | 1.886 | 2.920 | 4.303 | 6.965 | 9.925 | 31.599 |
| 3 | 0.765 | 0.978 | 1.250 | 1.638 | 2.353 | 3.182 | 4.541 | 5.841 | 12.924 |
| 4 | 0.741 | 0.941 | 1.190 | 1.533 | 2.132 | 2.776 | 3.747 | 4.604 | 8.610 |
| 5 | 0.727 | 0.920 | 1.156 | 1.476 | 2.015 | 2.571 | 3.365 | 4.032 | 6.869 |
| 6 | 0.718 | 0.906 | 1.134 | 1.440 | 1.943 | 2.447 | 3.143 | 3.707 | 5.959 |
| 7 | 0.711 | 0.896 | 1.119 | 1.415 | 1.895 | 2.365 | 2.998 | 3.499 | 5.408 |
| 8 | 0.706 | 0.889 | 1.108 | 1.397 | 1.860 | 2.306 | 2.896 | 3.355 | 5.041 |
| 9 | 0.703 | 0.883 | 1.100 | 1.383 | 1.833 | 2.262 | 2.821 | 3.250 | 4.781 |
| 10 | 0.700 | 0.879 | 1.093 | 1.372 | 1.812 | 2.228 | 2.764 | 3.169 | 4.587 |
| 11 | 0.697 | 0.876 | 1.088 | 1.363 | 1.796 | 2.201 | 2.718 | 3.106 | 4.437 |
| 12 | 0.695 | 0.873 | 1.083 | 1.356 | 1.782 | 2.179 | 2.681 | 3.055 | 4.318 |
| 13 | 0.694 | 0.870 | 1.079 | 1.350 | 1.771 | 2.160 | 2.650 | 3.012 | 4.221 |
| 14 | 0.692 | 0.868 | 1.076 | 1.345 | 1.761 | 2.145 | 2.624 | 2.977 | 4.140 |
| 15 | 0.691 | 0.866 | 1.074 | 1.341 | 1.753 | 2.131 | 2.602 | 2.947 | 4.073 |
| 16 | 0.690 | 0.865 | 1.071 | 1.337 | 1.746 | 2.120 | 2.583 | 2.921 | 4.015 |
| 17 | 0.689 | 0.863 | 1.069 | 1.333 | 1.740 | 2.110 | 2.567 | 2.898 | 3.965 |
| 18 | 0.688 | 0.862 | 1.067 | 1.330 | 1.734 | 2.101 | 2.552 | 2.878 | 3.922 |
| 19 | 0.688 | 0.861 | 1.066 | 1.328 | 1.729 | 2.093 | 2.539 | 2.861 | 3.883 |
| 20 | 0.687 | 0.860 | 1.064 | 1.325 | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| 21 | 0.686 | 0.859 | 1.063 | 1.323 | | | | | |
| 22 | 0.686 | 0.858 | 1.061 | 1.321 | | | | | |
| 23 | 0.685 | 0.858 | 1.060 | 1.319 | | | | | |
| 24 | 0.685 | 0.857 | 1.059 | 1.318 | | | | | |
| 25 | 0.684 | 0.856 | 1.058 | 1.316 | 1.708 | 2.060 | 2.485 | 2.787 | 3.725 |
| 26 | 0.684 | 0.856 | 1.058 | 1.315 | 1.706 | 2.056 | 2.479 | 2.779 | 3.707 |
| 27 | 0.684 | 0.855 | 1.057 | 1.314 | 1.703 | 2.052 | 2.473 | 2.771 | 3.690 |
| 28 | 0.683 | 0.855 | 1.056 | 1.313 | 1.701 | 2.048 | 2.467 | 2.763 | 3.674 |
| 29 | 0.683 | 0.854 | 1.055 | 1.311 | 1.699 | 2.045 | 2.462 | 2.756 | 3.659 |
| 30 | 0.683 | 0.854 | 1.055 | 1.310 | 1.697 | 2.042 | 2.457 | 2.750 | 3.646 |
| 40 | 0.681 | 0.851 | 1.050 | 1.303 | 1.684 | 2.021 | 2.423 | 2.704 | 3.551 |
| 50 | 0.679 | 0.849 | 1.047 | 1.299 | 1.676 | 2.009 | 2.403 | 2.678 | 3.496 |
| 100 | 0.677 | 0.845 | 1.042 | 1.290 | 1.660 | 1.984 | 2.364 | 2.626 | 3.390 |
| ∞ | 0.674 | 0.842 | 1.036 | 1.282 | 1.645 | 1.960 | 2.326 | 2.576 | 3.291 |

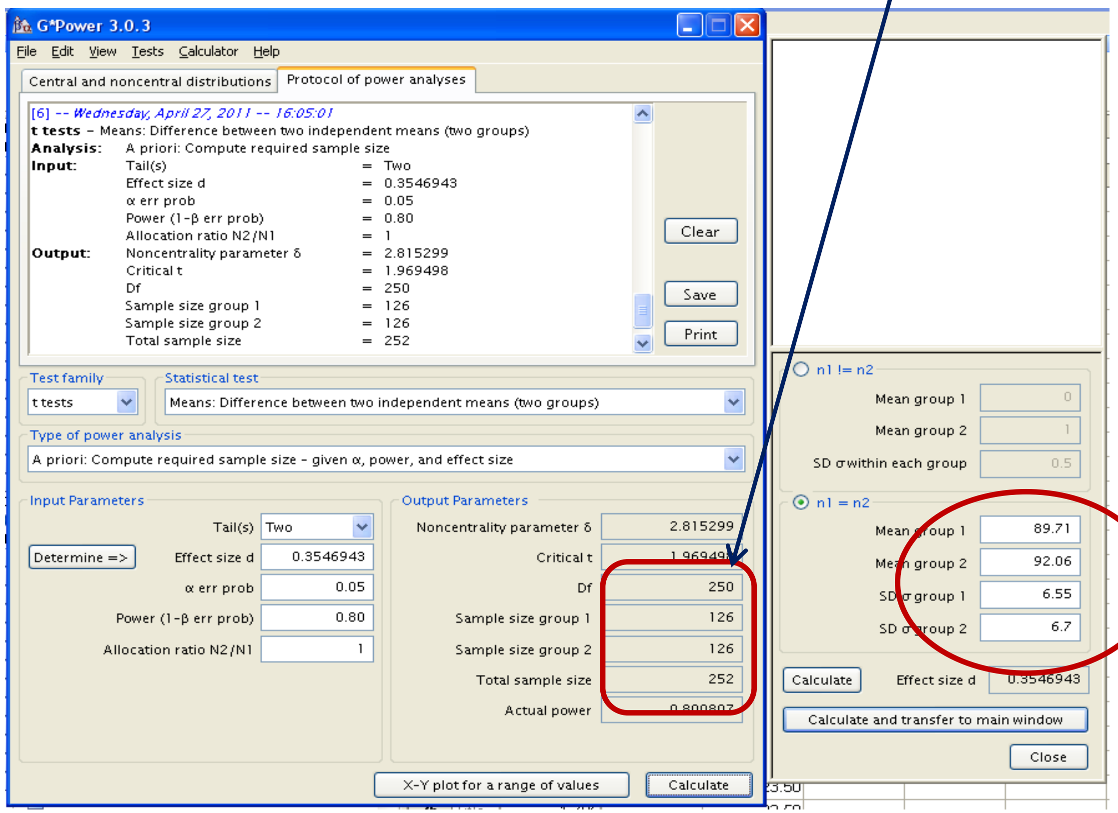
***t* = 1.641 < 1.984: not significant**

Critical value

**What about power?**

# Power analysis

You would need a sample 3 times bigger to reach the accepted power of 80%.




| | Col. stats | A<br>Females | B<br>Males |
|---|---|---|---|
| 1 | Number of values | 43 | 43 |
| 2 | | | |
| 3 | Minimum | 71.00 | 78.00 |
| 4 | 25% Percentile | 86.00 | 87.00 |
| 5 | Median | 90.00 | 92.00 |
| 6 | 75% Percentile | 93.50 | 96.00 |
| 7 | Maximum | 102.5 | 105.0 |
| 8 | | | |
| 9 | Mean | 89.71 | 92.06 |
| 10 | Std. Deviation | 6.550 | 6.696 |
| 11 | Std. Error of Mean | 0.9988 | 1.021 |
| 12 | | | |
| 13 | Lower 95% CI of mean | 87.70 | 90.00 |
| 14 | Upper 95% CI of mean | 91.73 | 94.12 |
| 15 | | | |
| 16 | Sum | 3858 | 3958 |
| 17 | | | |
| 18 | **D'Agostino & Pearson normality test** | | |
| 19 | K2 | 4.203 | 0.5080 |
| 20 | P value | 0.1223 | 0.7757 |
| 21 | Passed normality test (alpha=0.05)? | Yes | Yes |
| 22 | P value summary | ns | ns |
| 23 | | | |
| 24 | **Shapiro-Wilk normality test** | | |
| 25 | W | 0.9700 | 0.9845 |
| 26 | P value | 0.3164 | 0.8190 |
| 27 | Passed normality test (alpha=0.05)? | Yes | Yes |
| 28 | P value summary | ns | ns |

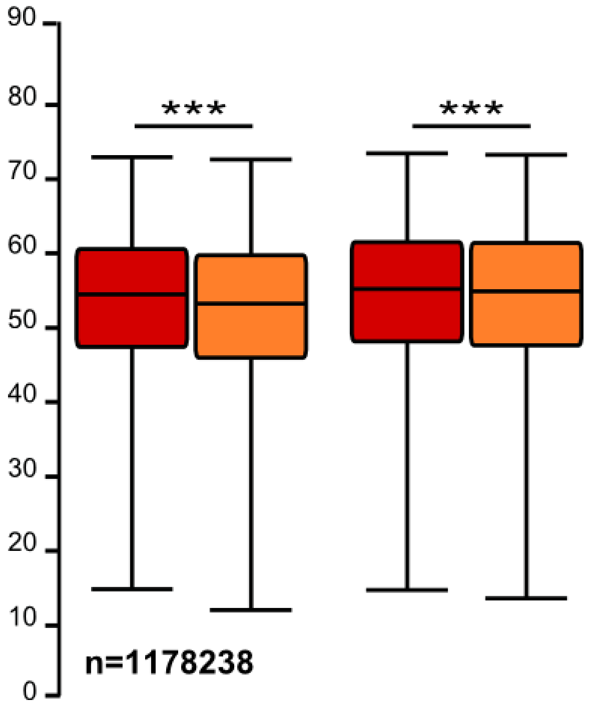
**But is a 2.3 cm difference between genders biologically relevant (<3%) ?**

# Sample size: the bigger the better?

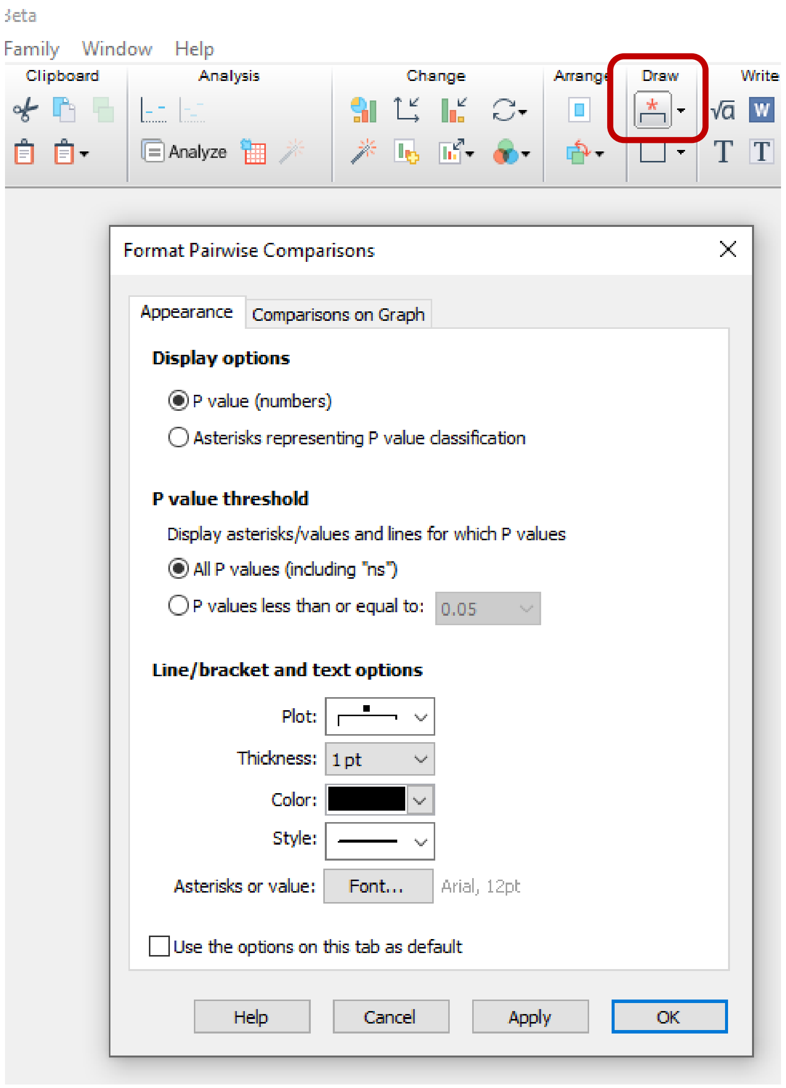
- It takes huge samples to detect tiny differences but tiny samples to detect huge differences.

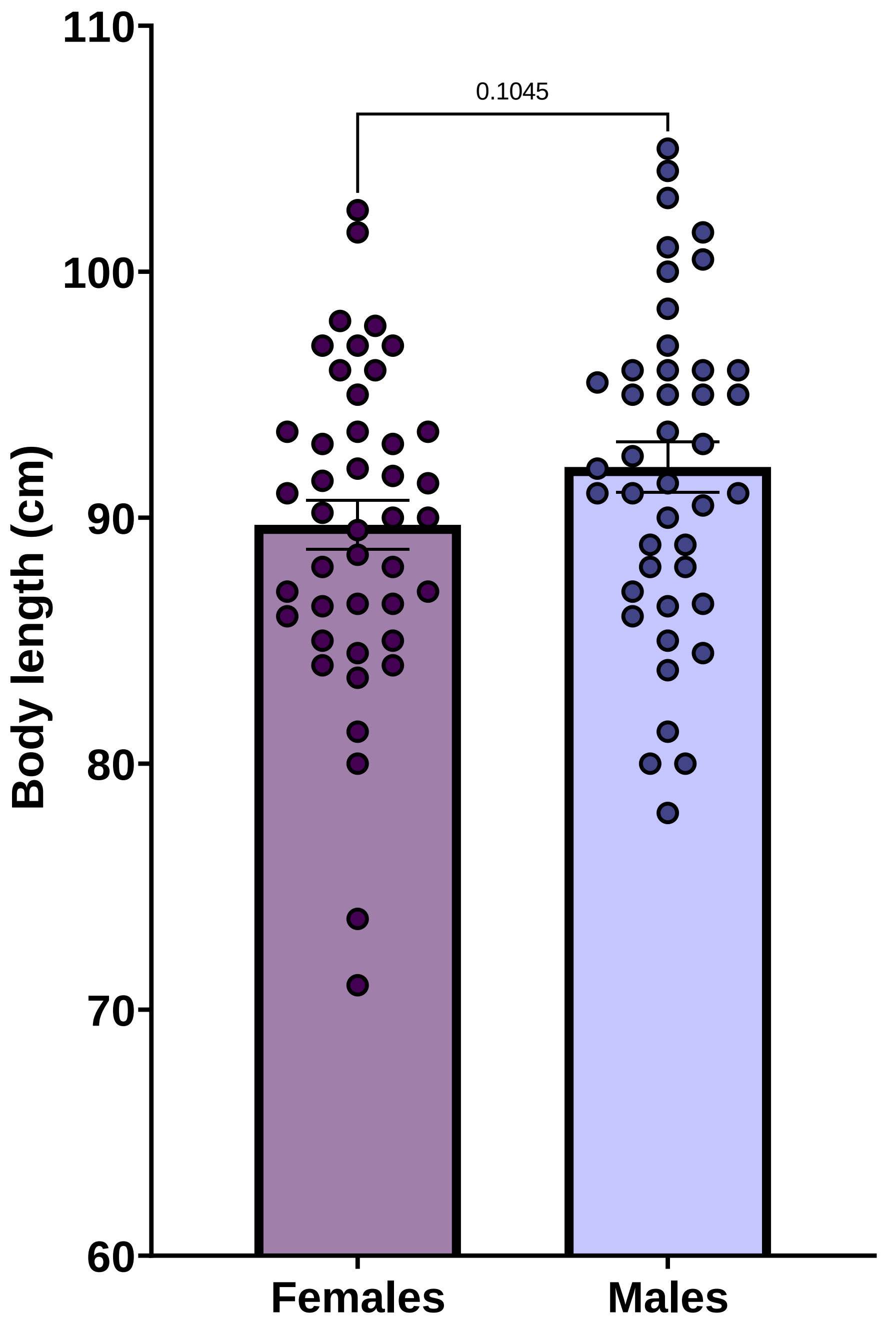
- What if the tiny difference is meaningless?
  - Beware of **overpower**
  - Nothing wrong with the stats: it is all about interpretation of the results of the test.

- Remember the important first step of power analysis
  - **What is the effect size of biological interest?**



# GraphPad Prism 9



## Exercise: Dependent or Paired *t*-test

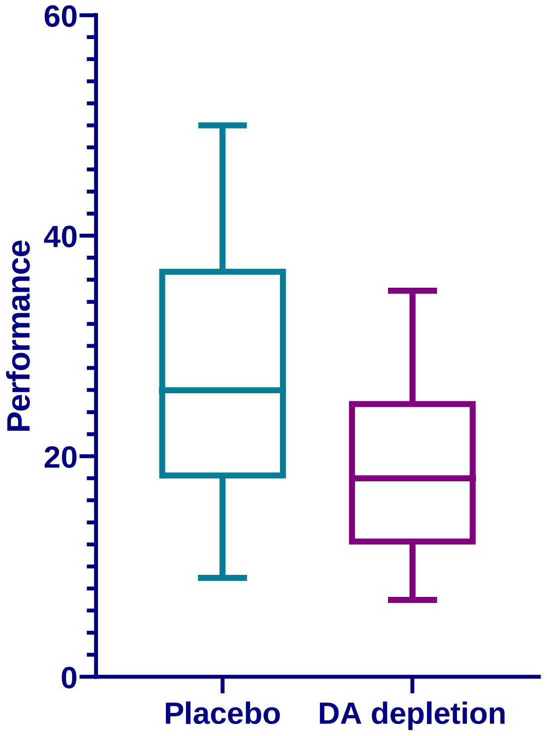
## working memory.xlsx

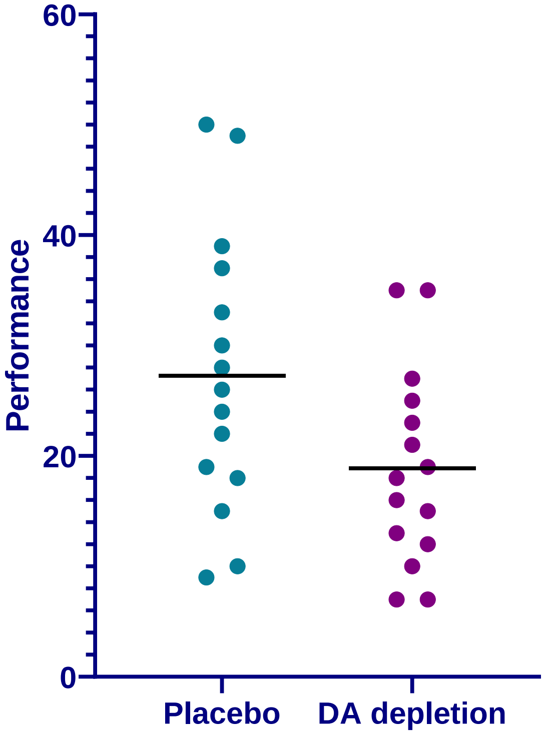
A group of rhesus monkeys (n=15) performs a task involving memory after having received a placebo. Their performance is graded on a scale from 0 to 100. They are then asked to perform the same task after having received a dopamine depleting agent.

Is there an effect of treatment on the monkeys' performance?

# Exercise: Dependent or Paired *t*-test

## working memory.xlsx



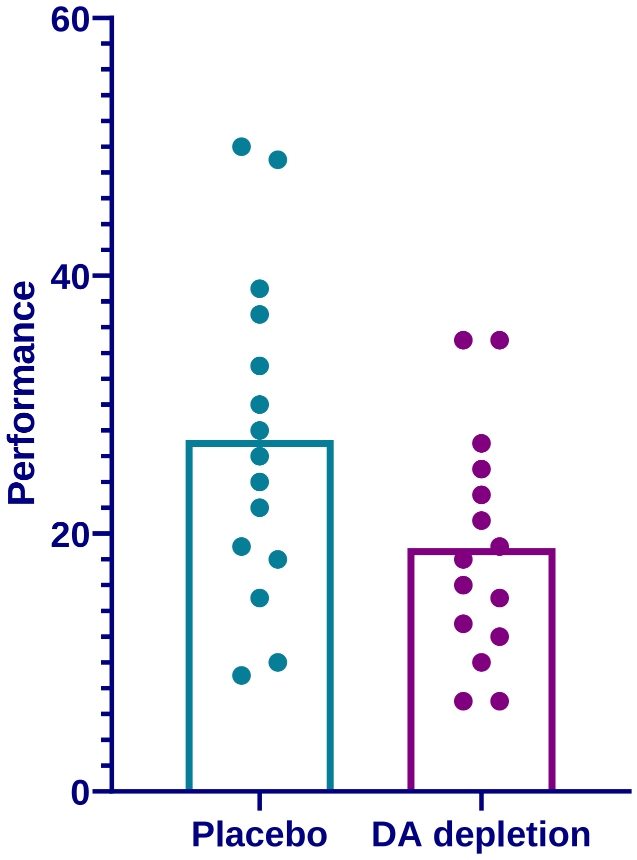




**Normaliy ☑**

# Exercise: Dependent or Paired *t*-test

## working memory.xlsx

| | Paired t test | |
|---|---|---|
| 1 | Table Analyzed | Working memory |
| 2 | | |
| 3 | Column A | Placebo |
| 4 | vs. | vs. |
| 5 | Column B | DA depletion |
| 6 | | |
| 7 | **Paired t test** | |
| 8 | P value | <0.0001 |
| 9 | P value summary | **** |
| 10 | Significantly different (P < 0.05)? | Yes |
| 11 | One- or two-tailed P value? | Two-tailed |
| 12 | t, df | t=8.616, df=14 |
| 13 | Number of pairs | 15 |
| 14 | | |
| 15 | **How big is the difference?** | |
| 16 | Mean of differences | 8.400 |
| 17 | SD of differences | 3.776 |
| 18 | SEM of differences | 0.9749 |
| 19 | 95% confidence interval | 6.309 to 10.49 |
| 20 | R squared (partial eta squared) | 0.8413 |
| 21 | | |
| 22 | **How effective was the pairing?** | |
| 23 | Correlation coefficient (r) | 0.9986 |
| 24 | P value (one tailed) | <0.0001 |
| 25 | P value summary | **** |
| 26 | Was the pairing significantly effective? | Yes |
| 27 | | |




# Paired *t*-test: Results

## working memory.xlsx